UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/761,530	01/21/2004	Dwight D. Koeberl	01579-1155	3856

23117 7590 05/24/2007

NIXON & VANDERHYE, PC
901 NORTH GLEBE ROAD, 11TH FLOOR
ARLINGTON, VA 22203

EXAMINER	
RAGHU, GANAPATHIRAM	
ART UNIT	PAPER NUMBER
1652	

MAIL DATE	DELIVERY MODE
05/24/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/761,530	KOEBERL ET AL.	
	Examiner	Art Unit	
	Ganapathirama Raghu	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on _____.

2a)☐ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-5,7-18 and 21-79* is/are pending in the application.

4a) Of the above claim(s) *30-72* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-5,7-18,21-29 and 73-79* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *03/19/07; 04/11/07*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☒ Other: *SEQ ALIGN*.

Application Status

Please note that the instant application/case has been transferred to examiner Ganapathirama Raghu, Art Unit 1652, whose telephone number is (571)-272-4533 and all further enquiries regarding this application should be directed to said examiner.

Applicants' response along with amendments to claims on 03/15/07 to the FOAM (09/15/06) by the previous examiner Charles Patterson is hereby acknowledged.

Claims 1-5, 7-18 and 21-79 are pending. Claims 30-72 remain withdrawn as they are directed to non-elected inventions. Thus, amended claims 1-5, 7-18, 21-29 and 73-79 are under consideration in the instant Office Action.

Objections and rejections not reiterated from the previous action are hereby withdrawn.

Priority

The priority date for the claims under consideration are assigned as follows: Claims 1-3, 5, 7, 10-18 and 21-29 are granted the priority date of Provisional Application No.: 60/441,789 filed on 01/22/2003. Claims 4, 8, 9 and 73-79 are only granted the priority date of Non-Provisional Application No.: 10/761,530 filed on 01/21/2004, as the subject matter recited in said claims were disclosed for the first time in said application.

Drawings

Drawings are accepted for examination purposes only.

Information Disclosure Statement

The information disclosure statement (IDS) submitted on 03/19/2007 and 04/11/2007, are in compliance with the provisions of 37 CFR 1.97. Accordingly, the examiner is considering the IDS statement.

Claim Rejections: 35 USC § 112-First Paragraph

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Enablement

Claim 1 and claims 2-5, 7-18, 21-29, 73-74 and 76-79 depending therefrom, are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for an isolated polynucleotide encoding a polypeptide of human acid-alpha-glucosidase (GAA), the mature form of said polypeptide comprising the amino acid sequence of SEQ ID NO: 2 encoded by a polynucleotide comprising the sequence of SEQ ID NO: 1, said GAA further comprising a 3' untranslated region of SEQ ID NO: 3 and a secretory signal sequence peptide selected from the group consisting of : SEQ ID NO: 5 (albumin), SEQ ID NO: 6 (erythropoietin), SEQ ID NO: 8 (human α-1-antitrypsin) or SEQ ID NO: 9 (Factor IX) and to a method for delivering said polypeptide through a vector comprising the encoded polynucleotides and a pharmaceutical composition comprising said vector. However the specification does not reasonably provide enablement for any isolated nucleic acid encoding a chimeric polypeptide comprising a secretory signal sequence operably linked to any human GAA polypeptide vector comprising said polynucleotides and a pharmaceutical composition comprising said vectors and to a method of delivering said polypeptide or (ii) any isolated nucleic acid encoding a chimeric polypeptide sequence operably linked any lysosomal polypeptide (as in claims 73, 74, 76, 78 and 79). The specification does not enable any person skilled in

the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with the claim.

Factors to be considered in determining whether undue experimentation is required are summarized in *In re Wands* (858 F.2d 731, 8 USPQ 2nd 1400 (Fed. Cir. 1988)) as follows: (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claim(s).

Claims 1-5, 7-18, 21-29, 73-74 and 76-79 are so broad as to encompass any isolated nucleic acid encoding a chimeric polypeptide comprising a secretory signal sequence operably linked to any human GAA polypeptide vector comprising said polynucleotides and a pharmaceutical composition comprising said vectors and to a method of delivering said polypeptide or (ii) any isolated nucleic acid encoding a chimeric polypeptide sequence operably linked any lysosomal polypeptide (as in claims 73, 74, 76, 78 and 79). The scope of the claims are not commensurate with the enablement provided by the disclosure with regard to the extremely large number of polynucleotides encoding any lysosomal polypeptide or any GAA broadly encompassed by the claims. Since the amino acid sequence of a protein encoded by a polynucleotide determines its structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires knowledge and guidance with regard to which amino acids in the protein's sequence and the respective codons in its polynucleotide, if any, are tolerant of modification and which are conserved (i.e. expectedly intolerant to modification), and detailed knowledge of the

ways in which the encoded proteins' structure relates to its function. In this case the disclosure is limited to an isolated polynucleotide encoding a polypeptide of human acid-alpha-glucosidase (GAA), the mature form of said polypeptide comprising the amino acid sequence of SEQ ID NO: 2 encoded by a polynucleotide comprising the sequence of SEQ ID NO: 1, said GAA further comprising a 3' untranslated region of SEQ ID NO: 3 and a secretory signal sequence peptide selected from the group consisting of: SEQ ID NO: 5 (albumin), SEQ ID NO: 6 (erythropoietin), SEQ ID NO: 8 (human α-1-antitrypsin) or SEQ ID NO: 9 (Factor IX) and to method for delivering said polypeptide through a vector comprising the encoded polynucleotides and a pharmaceutical composition comprising said vector. But the specification provides no guidance with regard to using variants, mutants and fragments thereof i. e., any isolated nucleic acid encoding a chimeric polypeptide comprising a secretory signal sequence operably linked to any human GAA polypeptide vector comprising said polynucleotides and a pharmaceutical composition comprising said vectors and to a method of delivering said polypeptide or (ii) any isolated nucleic acid encoding a chimeric polypeptide sequence operably linked any lysosomal polypeptide (as in claims 73, 74, 76, 78 and 79). In view of the great breadth of the claims, the amount of experimentation required to determine a use for the full scope of the claimed polypeptides, the lack of guidance, working examples, and unpredictability of the art in predicting function from a polypeptide primary structure (e.g., see Whisstock et al., Q Rev Biophys. 2003 Aug; 36(3): 307-340), the claimed invention would require undue experimentation. As such, the specification fails to teach one of ordinary skill how to use the full scope of the polypeptides encompassed by these claims.

While enzyme isolation techniques, recombinant and mutagenesis techniques are known, and it is not routine in the art to screen for multiple substitutions or multiple modifications as encompassed by the instant claims, the specific amino acid positions within a protein's sequence where amino acid modifications can be made with a reasonable expectation of success in obtaining the desired activity/utility are limited in any protein and the result of such modifications is unpredictable (e.g., see Whisstock et al., Q Rev Biophys. 2003 Aug; 36(3): 307-340). In addition, one skilled in the art would expect any tolerance to modification for a given protein to diminish with each further and additional modification, e.g. multiple substitutions or deletions.

The specification does not support the broad scope of the claims which encompasses any isolated nucleic acid encoding a chimeric polypeptide comprising a secretory signal sequence operably linked to any human GAA polypeptide vector comprising said polynucleotides and a pharmaceutical composition comprising said vectors and to a method of delivering said polypeptide or (ii) any isolated nucleic acid encoding a chimeric polypeptide sequence operably linked any lysosomal polypeptide (as in claims 73, 74, 76, 78 and 79). The specification does not enable the full scope of claims 1-5, 7-18, 21-29, 73-74 and 76-79, because the specification does not establish: (A) the structure of all polynucleotides and encoding polypeptides with desired acid alpha-glucosidase activity, including variants, mutants and recombinants or any polynucleotide encoding any lysosomal polypeptide with any activity including variants, mutants and recombinants; (B) regions of the polynucleotide/ protein structure which may be modified without affecting the activity of encoded polypeptide; (C) the general tolerance of the polynucleotide and the encoded polypeptide to modification and extent of

such tolerance; (D) a rational and predictable scheme for modifying any amino acid residue or the respective codon in the polynucleotide with an expectation of obtaining the desired biological function; and (E) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to use the claimed invention in a manner reasonably correlated with the scope of the claim broadly including polypeptides with an enormous number of modifications. The scope of the claim must bear a reasonable correlation with the scope of enablement (*In re Fisher*, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of targeted therapeutic polypeptide having the desired biological characteristics comprising any isolated nucleic acid encoding a chimeric polypeptide comprising a secretory signal sequence operably linked to any human GAA polypeptide vector comprising said polynucleotides and a pharmaceutical composition comprising said vectors and to a method of delivering said polypeptide or (ii) any isolated nucleic acid encoding a chimeric polypeptide sequence operably linked any lysosomal polypeptide (as in claims 73, 74, 76, 78 and 79), is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See *In re Wands* 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

Written Description

Claims 1-5, 7-18, 21-29, 73-74 and 76-79 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to

reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 1-5, 7-18, 21-29, 73-74 and 76-79, as interpreted, are directed to a genus of polynucleotides encoding a chimeric polypeptide comprising an amino acid sequence of human acid alpha-glucosidase (GAA) polypeptide including variants, mutants and recombinants or to a genus of polynucleotides encoding a chimeric polypeptide comprising any lysososmal polypeptide including variants, mutants and recombinants (as in claims 73, 74, 76, 78 and 79).

In *University of California v. Eli Lilly & Co.*, 43 USPQ2d 1938, the Court of Appeals for the Federal Circuit has held that "A written description of an invention involving a chemical genus, like a description of a chemical species, 'requires a precise definition, such as by structure, formula, [or] chemical name,' of the claimed subject matter sufficient to distinguish it from other materials". As indicated in MPEP § 2163, the written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show that Applicant was in possession of the claimed genus. In addition, MPEP § 2163 states that a representative number of species means that the species which are adequately described are representative of the entire genus. Thus, when there is substantial variation within the

genus, one must describe a sufficient variety of species to reflect the variation within the genus.

In the instant case, there is no structure correlated to associated function recited in claims with regard to the members of the genus of polynucleotides encoding a chimeric polypeptide comprising an amino acid sequence of human acid alpha-glucosidase (GAA) polypeptide including variants, mutants and recombinants as targeted therapeutic protein or to a genus of polynucleotides encoding a chimeric polypeptide comprising any lysososmal polypeptide including variants, mutants and recombinants. While the specification in the instant application discloses the structure of an isolated polynucleotide encoding a polypeptide of human acid-alpha-glucosidase (GAA), the mature form of said polypeptide comprising the amino acid sequence of SEQ ID NO: 2 encoded by a polynucleotide comprising the sequence of SEQ ID NO: 1, said GAA further comprising a 3' untranslated region of SEQ ID NO: 3 and a secretory signal sequence peptide selected from the group consisting of: SEQ ID NO: 5 (albumin), SEQ ID NO: 6 (erythropoietin), SEQ ID NO: 8 (human α-1-antitrypsin) or SEQ ID NO: 9 (Factor IX) and to method for delivering said polypeptide through a vector comprising the encoded polynucleotides and a pharmaceutical composition comprising said vector, it fails to provide any information as to the structure associated with function for the genus of polynucleotides claimed i.e., genus of polynucleotides encoding a chimeric polypeptide comprising an amino acid sequence of human acid alpha-glucosidase (GAA) polypeptide including variants, mutants and recombinants or to a genus of polynucleotides encoding a chimeric polypeptide comprising any lysososmal polypeptide

including variants, mutants and recombinants (as in claims 73, 74, 76, 78 and 79), with no structural limitations.

Due to the fact that the specification only discloses the structure an isolated polynucleotide encoding a polypeptide of human acid-alpha-glucosidase (GAA), the mature form of said polypeptide comprising the amino acid sequence of SEQ ID NO: 2 encoded by a polynucleotide comprising the sequence of SEQ ID NO: 1, said GAA further comprising a 3' untranslated region of SEQ ID NO: 3 and a secretory signal sequence peptide selected from the group consisting of: SEQ ID NO: 5 (albumin), SEQ ID NO: 6 (erythropoietin), SEQ ID NO: 8 (human α-1-antitrypsin) or SEQ ID NO: 9 (Factor IX) and to method for delivering said polypeptide through a vector comprising the encoded polynucleotides and a pharmaceutical composition comprising said vector, and the lack of description of any additional species/variants/mutants/recombinants by any relevant, identifying characteristics or properties, one of skill in the art would not recognize from the disclosure that Applicant was in possession of the claimed invention.

Applicant is referred to the revised guidelines concerning compliance with the written description requirement of U.S.C. 112, first paragraph, published in the Official Gazette and also available at www.uspto.gov.

Previous rejection dated 09/15/06 was mainly directed to scope of enablement, applicants' have traversed the rejection with the argument, the claims have been amended to overcome the rejections and also have requested to provide proper basis for the rejection or withdraw the same.

The applicants' traversal is answered as follows:

A new rejection detailing the lack of enablement and written description of the

disclosure is presented above. Regarding enablement, the scope and breadth of the claims encompass many polynucleotides encoding a chimeric polypeptide comprising any GAA or lysosomal polypeptide of any activity and from any source identified by only functional characteristics with no structural limitations. However, it is well known in the art that structurally related molecules may not possess similar function including desired specificity for substrates and enzyme kinetics and conversely functionally similar molecules may not share similar structural features or significant homology.

For example in the case of human GAA it has been shown in the art, even a single point mutation within the coding region of the polynucleotide can drastically affect the activity of the said polypeptide (see Hermans et al., 1991, 1992 and 1993; in IDS). Therefore a skilled artisan should be provided with the structural details of the polynucleotides and the encoded polypeptides, at least with respect to human GAA lysosomal polypeptide, as the prior art teaches that there are many naturally occurring variants (single nucleotide polymorphisms, SNPs) and the encoded polypeptides have wide ranging kinetic and biochemical characteristics. Similarly, for claims directed to polynucleotides encoding any lysosomal polypeptide with any activity or any chimeric lysosomal polypeptide there are no structural limitations. To further illustrate the importance of structure associated with correlated function, a few more examples of structure correlated to function are provided for the basis of rejection: Witkowski et al. (Biochemistry 38:11643-11650, 1999), teaches that one conservative amino acid substitution transforms a β-ketoacyl synthase into a malonyl decarboxylase and completely eliminates β-ketoacyl synthase activity. Seffernick et al. (J. Bacteriol. 183(8): 2405-2410, 2001), teaches that two naturally occurring *Pseudomonas* enzymes having

98% amino acid sequence identity catalyze two different reactions: deamination and dehalogenation, therefore having different function. Broun et al. (Science 282:1315-1317, 1998), teaches that as few as four amino acid substitutions can convert an oleate 12-desaturase into a hydrolase and as few as six amino acid substitutions can transform a hydrolase to a desaturase. Therefore, the claimed genera of polynucleotides encoding polypeptides include proteins having widely variable structures, since minor changes may result in changes affecting function and no additional information correlating structure with function has been provided. Many structurally unrelated polynucleotides and encoding polypeptides are encompassed by these claims. The specification only discloses a single species of the recited genus, which is insufficient to put one of ordinary skill in the art in possession of all attributes and features of all species within the required genus. Therefore, one skilled in the art cannot reasonably conclude that the applicant had possession of the claimed invention at the time the instant application was filed.

Claim Rejections 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless -

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1-2, 5, 7-18 and 21-29 are rejected under 35 U.S.C. 102(b) as being anticipated by Amalfitano et al., (WO 02/098466 A1, 2002, in IDS) when given the broadest interpretation. Claims 1-2, 5, 7-18 and 21-29 are directed to any isolated nucleic acid encoding a chimeric polypeptide comprising a secretory signal sequence, i. e., any human GAA polypeptide and comprising any secretory signal sequence including

variants, mutants and recombinants and further comprising a polynucleotide from any 3' untranslated region, vector comprising said polynucleotides and a pharmaceutical composition comprising said vectors and to a method of delivering said polypeptide. Amalfitano et al., (*supra*) teach adenovirus and adeno-associated virus vectors comprising polynucleotides encoding chimeric polypeptides comprising a secretory signal sequence operably linked to human GAA (lines 13-26, page 22) and a method of producing said polypeptide in many mammalian cultured cells such as CHO, 293 and in vivo in heaptocytes (Summary of the Invention: pages 3-41; especially pages 6, 7, 12, 22, 26, 28-30, 35, 41 and Examples 1, 4, 9, and 13). Therefore, the reference of Amalfitano et al., anticipates claims 1-2, 5, 7-18 and 21-29 of the present invention.

Claims 1-2, 5, 7-11, 14, 15, 17 and 21-29 are rejected under 35 U.S.C. 102(b) as being anticipated by Van Bree et al., (WO 00/34451, 2000, in IDS) when given the broadest interpretation. Claims 1-2, 5, 7-11, 14, 15, 17 and 21-29 are directed to any isolated nucleic acid encoding a chimeric polypeptide comprising a secretory signal sequence, i. e., any human GAA polypeptide and comprising any secretory signal sequence including variants, mutants and recombinants and further comprising a polynucleotide from any 3' untranslated region, vector comprising said polynucleotides and a pharmaceutical composition comprising said vectors and to a method of delivering said polypeptide. Van Bree et al., (*supra*) teach compositions comprising polynucleotides encoding the human GAA with native secretory signal sequence and also suggest said GAA can be operably linked to other signal peptides (page 9, lines 16-30), vectors, methods of expression, pharmaceutical composition comprising said polynucleotides and

encoded polypeptides, and a method of producing said polypeptide in many mammalian cultured cells such as CHO, 293 and in vivo in heaptocytes, method of administering said compositions to treat Pompe's disease (GAA deficiency) and methods to generate transgenic animals comprising polynucleotides encoding human GAA (Summary of the Invention: pages 3-28; especially pages 7, 9 and 10). Therefore, the reference of Van Bree et al., anticipates claims 1-2, 5, 7-11, 14, 15, 17 and 21-29 of the present invention.

Claim Rejections 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 3-4, 73-75 and 77-79 are rejected under 35 U.S.C. 103(a) as being anticipated by Amalfitano et al., (WO 02/098466 A1, 2002, in IDS) in view of Heus JH (US Patent No.: 6,858,425 B1, claiming priority date of Application No.: 09/454,466 filed on 12/03/99) and Haseltine et al., (WO 2005/003296 A2, claiming priority date of

Application No.: 60/441,305 filed on 01/22/03). Rejection of claims 1-2, 5, 7-18 and 21-29 under 35 U.S.C. 102(b) as being anticipated by Amalfitano et al., (WO 02/098466 A1, 2002, in IDS) is discussed above. Amalfitano et al., teach isolated nucleic acids expressing lysosomal polypeptides a chimeric polypeptide comprising secretory signal sequence operably linked to human acid alpha-glucosidase (GAA), the full-length polypeptide, cleaved mature forms of polypeptides including chimeric polypeptides comprising secretory signal sequences operably linked to said polypeptides), heterologous sequences (operably linked to the polynucleotide). Amalfitano et al., is silent regarding said polynucleotide comprising the 3'untrnasalted region of SEQ ID NO: 3 or said polynucleotide encoding a fusion polypeptide comprising SEQ ID NO: 5, an albumin signal peptide sequence. Heus JH et al., have disclosed the human alpha gluosidase (GAA) gene, vector constructs and the 3'untranslated region sequence of said gene (entire document). Haseltine et al., teach the albumin signal peptide sequence of SEQ ID NO: 5 and methods for fusing said signal peptide sequence linked to various therapeutic proteins as fusion proteins for use in gene therapy techniques. Therefore, it would have been obvious to a person of ordinary skill in the art to combine the teachings of Amalfitano et al., Heus JH., and Haseltine et al., to produce an isolated nucleic acid encoding a chimeric therapeutic polypeptide such as human GAA comprising the 3'untranslated region of the human GAA polynucleotide sequence and further said encoded chimeric polypeptide comprising the albumin secretory signal peptide. Motivation to generate a therapeutic GAA comprising a secretory signal peptide derives from the fact that therapeutic lysosomal enzyme polypeptides are endowed with properties that enable them to be efficiently targeted to sub-cellular compartments such as

endoplasmic reticulum and golgi apparatus for post-translational modification of said therapeutic proteins and for efficient processing and transport in the target tissues. The expectation of success is high, because Amalfitano et al., teach the use of lysosomal polypeptides such as human acid alpha-glucosidase (GAA), distinct advantages and the method of use of said polypeptide for therapeutic purposes and Heus JH., and Haseltine et al., teach the use of 3'untranslated region of the human GAA polynucleotide sequence and albumin secretory signal peptide as a chimeric polypeptide for effective targeting of said therapeutic polypeptides to clinically significant tissues. Therefore, claims 3-4, 73-75 and 77-79 are rejected under 35 U.S.C. 103(a) as being anticipated by Amalfitano et al., (WO 02/098466 A1, 2002, in IDS) in view of Heus JH (US Patent No.: 6,858,425 B1, claiming priority date of Application No.: 09/454,466 filed on 12/03/99) and Haseltine et al., (WO 2005/003296 A2, claiming priority date of Application No.: 60/441,305 filed on 01/22/03).

Claims 1-3, 5, 7-18, 21-29, 73-75 and 77-79 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Amalfitano et al., and Heus JH and further in view of Martin et al., (WO 00/47741, 2000). Amalfitano et al., and Heus JH are described above. Said references do not specifically teach encoded chimeric polypeptide comprising an erythropoietin secretory signal sequence of SEQ ID NO: 6 linked to human GAA. Martin et al., specifically teach a therapeutic polypeptide comprising a native human erythropoietin signal peptide of SEQ ID NO: 6 (entire document). It would have been obvious to a person of ordinary skill in the art to combine the teachings of Amalfitano et al., Heus JH, and Martin et al., to produce a targeted therapeutic glycoprotein with a an erythropoietin secretory signal sequence linked to therapeutic

polypeptide GAA. Motivation to generate a therapeutic GAA comprising a secretory signal peptide derives from the fact that therapeutic lysosomal enzyme polypeptides are endowed with properties that enable them to be efficiently targeted to sub-cellular compartments such as endoplasmic reticulum and golgi apparatus for post-translational modification of said therapeutic proteins and for efficient processing and transport in the target tissues. The expectation of success is high, because Martin et al., teach the utility of therapeutic polypeptides comprising an erythropoietin secretory signal for effective targeting of said therapeutic polypeptides to clinically significant tissues. Therefore, claims 1-3, 5, 7-18, 21-29, 73-75 and 77-79 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Amalfitano et al., and Heus JH and further in view of Martin et al., (WO 00/47741, 2000).

Claims 1-3, 5, 7-18, 21-29 and 73-79 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Amalfitano et al., and Heus JH and further in view of Whitfeld et al., (US Patent No.: 5,298,400, 1994). Amalfitano et al., and Heus JH are described above. Said references do not specifically teach encoded chimeric polypeptide comprising a α-1-antitrypsin secretory signal sequence of SEQ ID NO: 8 linked to human GAA. Whitfeld et al., specifically teach a therapeutic polypeptide comprising a α-1-antitrypsin secretory signal sequence of SEQ ID NO: 8 (entire document). It would have been obvious to a person of ordinary skill in the art to combine the teachings of Amalfitano et al., Heus JH, and Whitfeld et al., to produce a targeted therapeutic glycoprotein with a an α-1-antitrypsin secretory signal sequence linked to therapeutic polypeptide GAA. Motivation to generate a therapeutic GAA comprising a secretory signal peptide derives from the fact that therapeutic lysosomal enzyme

polypeptides are endowed with properties that enable them to be efficiently targeted to sub-cellular compartments such as endoplasmic reticulum and golgi apparatus for post-translational modification of said therapeutic proteins and for efficient processing and transport in the target tissues. The expectation of success is high, because Whitfeld et al., teach the utility of therapeutic polypeptides comprising a α-1-antitrypsin secretory signal for effective targeting of said therapeutic polypeptides to clinically significant tissues. Therefore, claims 1-3, 5, 7-18, 21-29 and 73-79 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Amalfitano et al., and Heus JH and further in view of Whitfeld et al., (US Patent No.: 5,298,400, 1994).

Claims 1-3, 5, 7-18, 21-29 and 73-79 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Amalfitano et al., and Heus and further in view of Meulien P (US Patent No.: 5,521,070, 1996). Amalfitano et al., and Heus JH are described above. Said references do not specifically teach encoded chimeric polypeptide comprising a Factor IX secretory signal sequence of SEQ ID NO: 9 linked to human GAA. Meulien P specifically teaches a therapeutic polypeptide comprising a Factor IX secretory signal sequence of SEQ ID NO: 9 (entire document). It would have been obvious to a person of ordinary skill in the art to combine the teachings of Amalfitano et al., Heus JH, and Meulien P to produce a targeted therapeutic glycoprotein with a Factor IX secretory signal sequence linked to therapeutic polypeptide GAA. Motivation to generate a therapeutic GAA comprising a secretory signal peptide derives from the fact that therapeutic lysosomal enzyme polypeptides are endowed with properties that enable them to be efficiently targeted to sub-cellular compartments such as endoplasmic reticulum and golgi apparatus for post-translational modification of said therapeutic

proteins and for efficient processing and transport in the target tissues. The expectation of success is high, because Meulien P et al., teach the utility of therapeutic polypeptides comprising a Factor IX secretory signal for effective targeting of said therapeutic polypeptides to clinically significant tissues. Therefore, claims 1-3, 5, 7-18, 21-29 and 73-79 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Amalfitano et al., and Heus and further in view of Meulien P (US Patent No.: 5,521,070, 1996).

Therefore, the above references render claims 1-5, 7-18, 21-29 and 73-79 *prima facie* obvious to one of ordinary skill in the art.

Applicants have traversed the previous rejections of claims 1-5, 7-18 and 21-29 under 103(a) as being unpatentable over McCown et al., and Barash et al., said rejection is being withdrawn, as applicants' have amended said claims and have added a new set of claims 73-79.

Summary of Pending Issues

The following is a summary of issues pending in the instant application.

1. Claims 1-5, 7-18, 21-29, 73-74 and 76-79 are rejected under 35 U.S.C. 112, first paragraph, for enablement and written description.

2. Claims 1-2, 5, 7-18 and 21-29 are rejected under 35 U.S.C. 102(b) as being anticipated by Amalfitano et al., (WO 02/098466 A1, 2002, in IDS).

3. Claims 1-2, 5, 7-11, 14, 15, 17 and 21-29 are rejected under 35 U.S.C. 102(b) as being anticipated by Van Bree et al., (WO 00/34451, 2000, in IDS).

4. Claims 1-5, 7-18, 21-29 and 73-79 *prima facie* obvious Amalfitano et al., (WO 02/098466 A1, 2002, in IDS) in view of Heus JH (US Patent No.: 6,858,425 B1,

claiming priority date of Application No.: 09/454,466 filed on 12/03/99), Haseltine et al., (WO 2005/003296 A2, claiming priority date of Application No.: 60/441,305 filed on 01/22/03), Martin et al., (WO 00/47741, 2000), Whitfeld et al., (US Patent No.: 5,298,400, 1994) and Meulien P (US Patent No.: 5,521,070, 1996).

Allowable Subject Matter/Conclusion

None of the claims are allowable.

Final Comments

To insure that each document is properly filed in the electronic file wrapper, it is requested that each of amendments to the specification, amendments to the claims, Applicants' remarks, requests for extension of time, and any other distinct papers be submitted on separate pages.

It is also requested that Applicants identify support, within the original application, for any amendments to the claims and specification.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Ganapathirama Raghu whose telephone number is 571-272-4533. The examiner can normally be reached on M-F; 8:00-4:30 pm EST. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapu Achutamurthy can be reached on 571-272-0928. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300 for regular communications and for After Final communications. Any inquiry of a general nature or relating to the status of the application or proceeding should be directed to the receptionist whose telephone number is 571-272-1600.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Ganapathirama Raghu, Ph.D.
Patent Examiner
Art Unit 1652
May 13, 2007.

Rebecca Prouty
REBECCA E. PROUTY
PRIMARY EXAMINER
GROUP 1600

SEQ ID NO: 3

<!--StartFragment-->RESULT 5
US-09-454-466-1
; Sequence 1, Application US/09454466
; Patent No. 6858425
; GENERAL INFORMATION:
; APPLICANT: Heus, Joris Jan
; APPLICANT: Pharming Intellectual Property B.V.
; TITLE OF INVENTION: HUMAN ACID ALPHA GLUCOSIDASE GENE AND BOVINE ALPHA-S1
; TITLE OF INVENTION: CASEIN GENE SEQUENCES
; FILE REFERENCE: 016994-013720US
; CURRENT APPLICATION NUMBER: US/09/454,466
; CURRENT FILING DATE: 1999-12-03
; EARLIER APPLICATION NUMBER: 60/110,859
; EARLIER FILING DATE: 1998-12-04
; EARLIER APPLICATION NUMBER: 60/122,550
; EARLIER FILING DATE: 1999-03-02
; NUMBER OF SEQ ID NOS: 5
; SOFTWARE: PatentIn Ver. 2.1
; SEQ ID NO 1
; LENGTH: 26167
; TYPE: DNA
; ORGANISM: Homo sapiens
US-09-454-466-1

Query Match 81.5%; Score 110; DB 3; Length 26167;
Best Local Similarity 100.0%; Pred. No. 8.8e-25;
Matches 110; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

```
Qy          1 TCAGCTGGTGTTAGCCGGGCGGAGTGTGTTAGTCTCTCCAGAGGGAGGCTGGTTCCCCAG 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      18631 TCAGCTGGTGTTAGCCGGGCGGAGTGTGTTAGTCTCTCCAGAGGGAGGCTGGTTCCCCAG 18690

Qy         61 GGAAGCAGAGCCTGTGTGCGGGCAGCAGCTGTGTGCGGGCCTGGGGGTTG 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db      18691 GGAAGCAGAGCCTGTGTGCGGGCAGCAGCTGTGTGCGGGCCTGGGGGTTG 18740
```

<!--EndFragment-->

US006858425B1

(12) **United States Patent**
Heus

(10) **Patent No.: US 6,858,425 B1**
(45) **Date of Patent: Feb. 22, 2005**

(54) **HUMAN ACID ALPHA GLUCOSIDASE GENE AND BOVINE ALPHA-S1 CASEIN GENE SEQUENCES**

(75) Inventor: **Joris Jan Heus**, Amsterdam (NL)

(73) Assignee: **Genzyme Corporation**, Cambridge, MA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/454,466**

(22) Filed: **Dec. 3, 1999**

Related U.S. Application Data

(60) Provisional application No. 60/122,550, filed on Mar. 2, 1999, and provisional application No. 60/110,850, filed on Dec. 4, 1998.

(51) **Int. Cl.**[7] **C07H 21/04**; C12N 9/00; C12N 15/00; C12N 9/36

(52) **U.S. Cl.** ..... **435/320.1**; 435/4; 435/6; 435/69.1; 435/183; 435/195; 435/206; 435/252.1; 536/23.2; 536/24.1 T

(58) **Field of Search** ..... 435/4, 6, 69.1, 435/183, 195, 206, 252.1, 320.1; 536/23.2, 24.1 T, 23.1; 702/520

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,565,334 A * 10/1996 Abe et al.

FOREIGN PATENT DOCUMENTS

WO WO94/16057 A * 7/1994

OTHER PUBLICATIONS

Tzall et al. Identification of the promoter region and gene expression for human acid alpha glucosidase. Biochem. Biophys. Res. Comm., vol. 176(3):1509–1515, 1991.*

GenBank Accession No. X55079, Hoefsloot et al. dated Nov. 14, 1998.*

GenBank Accession No. X59856, Koczan et al. dated Oct. 24, 1991.*

GenBank Accession No. Q66990, Abe et al. dated Jul. 21, 1994.*

* cited by examiner

Primary Examiner—Manjunath N. Rao
(74) *Attorney, Agent, or Firm*—Townsend and Townsend and Crew LLP

(57) **ABSTRACT**

The invention provides polynucleotide sequences from the human acid alpha glucosidase gene and the bovine alpha S1 casein gene. These sequences are useful for designing transgenes for expression of human acid alpha glucosidase in the milk of transgenic animals. The sequences are also useful for design of primers and probes, and for computerized methods of sequence comparison.

5 Claims, 9 Drawing Sheets

SEQ ID NO: 3

SE

SEQ ID NO: 5 Albumin fusion with signal replica

<!--StartFragment-->RESULT 2
ADW45546
ID ADW45546 standard; peptide; 21 AA.
XX
AC ADW45546;
XX
DT 07-APR-2005 (first entry)
XX
DE Fusion protein-related consensus signal peptide 2.
XX
KW fusion protein; anti-HIV; gastrointestinal-gen.; antidiabetic; anorectic;
KW nephrotropic; cardiant; cytostatic; neuroprotective; immunosuppressive;
KW immune disorder; hematological disease; hyperproliferative disorder;
KW renal disease; cardiovascular disease; cardiovascular-gen.;
KW respiratory disorder; angiogenesis disorder; neurological disease;
KW wound healing; vulnerary; endocrine disease; reproductive disorder;
KW gynecological; infectious disease; antimicrobial;
KW gastrointestinal disease; gene therapy.
XX
OS Unidentified.
XX
PN WO2005003296-A2.
XX
PD 13-JAN-2005.
XX
PF 20-JAN-2004; 2004WO-US001369.
XX
PR 22-JAN-2003; 2003US-0441305P.
PR 11-MAR-2003; 2003US-0453201P.
PR 02-MAY-2003; 2003US-0467222P.
PR 23-MAY-2003; 2003US-0472816P.
PR 06-JUN-2003; 2003US-0476267P.
PR 24-SEP-2003; 2003US-0505172P.
PR 30-SEP-2003; 2003US-0506746P.
XX
PA (HUMA-) HUMAN GENOME SCI INC.
XX
PI Haseltine WA, Rosen CA;
XX
DR WPI; 2005-091786/10.
XX
PT New albumin fusion protein for diagnosing, treating or preventing
PT diseases such as HIV/AIDS, diabetes, obesity, heart disease or immune
PT disorders comprises a therapeutic protein (e.g. CD4M33, GLP-2 or PACAP-
PT 27) and an albumin.
XX
PS Disclosure; SEQ ID NO 550; 884pp; English.
XX
CC The invention relates to a novel albumin fusion protein comprising a
CC therapeutic protein as listed in the specification in Table 1 and an
CC albumin comprising a sequence of SEQ ID NO: 1, or a fragment or variant
CC of SEQ ID NO: 1, where the fragment or variant has albumin activity and
CC where the albumin activity is the ability to prolong the shelf life of
CC the therapeutic protein compared to the shelf-life of the therapeutic
CC protein in an unfused state. Human serum albumin (HSA, HA) is responsible
CC for a significant proportion of the osmotic pressure of serum and also
CC functions as a carrier of endogenous and exogenous ligands. The fusion
CC protein of the invention demonstrates anti-HIV, gastrointestinal-gen.,
CC antidiabetic, anorectic, cardiant and immunosuppressive activities. The
CC fusion protein may be useful for diagnosing, treating, preventing or
CC ameliorating diseases, such as immune disorders, blood disorders,

```
CC   hyperproliferative disorders, renal disorders, cardiovascular disorders,
CC   respiratory disorders, angiogenesis-related disorders, neurological
CC   disorders, wound healing disorders, endocrine disorders, reproductive
CC   disorders, infectious disorders and gastrointestinal disorders, possibly
CC   with the use of gene therapy techniques. The current sequence is that of
CC   the fusion protein-related consensus signal peptide 2.of the invention.
XX
SQ   Sequence 21 AA;

  Query Match             100.0%;  Score 132;  DB 9;  Length 21;
  Best Local Similarity   100.0%;  Pred. No. 1e-08;
  Matches   21;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 MWWRLWWLLLLLLLLWPMVWA 21
              |||||||||||||||||||||
Db          1 MWWRLWWLLLLLLLLWPMVWA 21

<!--EndFragment-->
```

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
13 January 2005 (13.01.2005)

PCT

(10) International Publication Number
WO 2005/003296 A2

(51) International Patent Classification[7]: C12N

(21) International Application Number:
PCT/US2004/001369

(22) International Filing Date: 20 January 2004 (20.01.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/441,305	22 January 2003 (22.01.2003)	US
60/453,201	11 March 2003 (11.03.2003)	US
60/467,222	2 May 2003 (02.05.2003)	US
60/472,816	23 May 2003 (23.05.2003)	US
60/476,267	6 June 2003 (06.06.2003)	US
60/505,172	24 September 2003 (24.09.2003)	US
60/506,746	30 September 2003 (30.09.2003)	US

(71) Applicant *(for all designated States except US)*: **HUMAN GENOME SCIENCES, INC.** [US/US]; 14200 Shady Grove Road, Rockville, MD 20850 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **HASELTINE, William, A.** [US/US]; 3053 P Street, N.W., Washington, DC 20007 (US). **ROSEN, Craig, A.** [US/US]; 22400 Rolling Hill Lane, Laytonsville, MD 20882 (US).

(74) Agents: **HOOVER?, Kenley, K.?** et al.; 14200 Shady Grove Road, Rockville, MD 20850 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK,

[Continued on next page]

(54) Title: ALBUMIN FUSION PROTEINS

```
  1 GAT GCA CAC AAG AGT GAG GTT GCT CAT CGG TTT AAA GAT TTG GGA GAA GAA AAT TTC AAA 60
  1 D   A   H   K   S   E   V   A   H   R   F   K   D   L   G   E   E   N   F   K   20

 61 GCC TTG GTG TTG ATT GCC TTT GCT CAG TAT CTT CAG CAG TGT CCA TTT GAA GAT CAT GTA 120
 21 A   L   V   L   I   A   F   A   Q   Y   L   Q   Q   C   P   F   E   D   H   V   40

121 AAA TTA GTG AAT GAA GTA ACT GAA TTT GCA AAA ACA TGT GTT GCT GAT GAG TCA GCT GAA 180
 41 K   L   V   N   E   V   T   E   F   A   K   T   C   V   A   D   E   S   A   E   60

181 AAT TGT GAC AAA TCA CTT CAT ACC CTT TTT GGA GAC AAA TTA TGC ACA GTT GCA ACT CTT 240
 61 N   C   D   K   S   L   H   T   L   F   G   D   K   L   C   T   V   A   T   L   80

241 CGT GAA ACC TAT GGT GAA ATG GCT GAC TGC TGT GCA AAA CAA GAA CCT GAG AGA AAT GAA 300
 81 R   E   T   Y   G   E   M   A   D   C   C   A   K   Q   E   P   E   R   N   E   100

301 TGC TTC TTG CAA CAC AAA GAT GAC AAC CCA AAC CTC CCC CGA TTG GTG AGA CCA GAG GTT 360
101 C   F   L   Q   H   K   D   D   N   P   N   L   P   R   L   V   R   P   E   V   120

361 GAT GTG ATG TGC ACT GCT TTT CAT GAC AAT GAA GAG ACA TTT TTG AAA AAA TAC TTA TAT 420
121 D   V   M   C   T   A   F   H   D   N   E   E   T   F   L   K   K   Y   L   Y   140

421 GAA ATT GCC AGA AGA CAT CCT TAC TTT TAT GCC CCG GAA CTC CTT TTC TTT GCT AAA AGG 480
141 E   I   A   R   R   H   P   Y   F   Y   A   P   E   L   L   F   F   A   K   R   160
```

(57) **Abstract:** The present invention encompasses albumin fusion proteins. Nucleic acid molecules encoding the albumin fusion proteins of the invention are also encompassed by the invention, as are vectors containing these nucleic acids, host cells transformed with these nucleic acids vectors, and methods of making the albumin fusion proteins of the invention and using these nucleic acids, vectors, and/or host cells. Additionally the present invention encompasses pharmaceutical compositions comprising albumin fusion proteins and methods of treating, preventing, or ameliorating diseases, disordrs or conditions using albumin fusion proteins of the invention.

WO 2005/003296 A2

SEQ ID NO: 6
Erythropoietin

<!--StartFragment-->RESULT 1
AAY95835
ID AAY95835 standard; peptide; 27 AA.
XX
AC AAY95835;
XX
DT 07-NOV-2000 (first entry)
XX
DE Native human erythropoietin signal peptide.
XX
KW Leptin; human; glycosylation; obesity; diabetes; hyperlipidemia;
KW antiobesity; antidiabetic; hyperlipemic; therapy; signal peptide;
KW erythropoietin.
XX
OS Homo sapiens.
XX
PN WO200047741-A1.
XX
PD 17-AUG-2000.
XX
PF 11-FEB-2000; 2000WO-US003652.
XX
PR 12-FEB-1999; 99US-00249675.
XX
PA (AMGE-) AMGEN INC.
XX
PI Martin FH, Elliott SG;
XX
DR WPI; 2000-524540/47.
XX
PT Glycosylated leptin proteins having a Stokes' radius greater than that of
PT a naturally occurring glycosylated human leptin useful for treating
PT obesity, diabetes and the effects of high blood lipid content.
XX
PS Example 14; Page 100; 156pp; English.
XX
CC The present sequence is that of the native human erythropoietin signal
CC peptide. The invention is directed to glycosylated leptin proteins (see
CC AAY95799-804) that have a Stokes' radius greater than that of naturally
CC occurring human leptin. A claimed method for manufacturing a glycosylated
CC leptin involves culturing a host cell containing a DNA sequence encoding
CC a signal peptide and a glycosylated leptin protein. Preferred signal
CC peptides have a peptidase cleavage site optimized for glycosylation
CC efficiency. When leptin+47+69+102 (see AAY95802) was expressed as a
CC fusion with the present signal peptide, the degree of glycosylation (on a
CC scale of 1-5) was 3 in COS host cells and 1.5 in CHO cells. Glycosylated
CC leptins, or nucleic acids encoding them, are used in the treatment of
CC obesity, diabetes and the effects of high blood lipid content (claimed).
CC They have longer systemic circulation times in vivo than native leptins
XX
SQ Sequence 27 AA;

Query Match 100.0%; Score 148; DB 3; Length 27;
Best Local Similarity 100.0%; Pred. No. 3.6e-13;
Matches 27; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

Qy 1 MGVHECPAWLWLLLSLLSLPLGLPVLG 27
||||||||||||||||||||||||||||
Db 1 MGVHECPAWLWLLLSLLSLPLGLPVLG 27

<!--EndFragment-->

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C12N 15/16, C07K 14/575, C12N 15/63, 5/10, A61K 38/22, A61P 3/04, 3/06, 5/48, C07K 16/26

A1

(11) International Publication Number: WO 00/47741

(43) International Publication Date: 17 August 2000 (17.08.00)

(21) International Application Number: PCT/US00/03652

(22) International Filing Date: 11 February 2000 (11.02.00)

(30) Priority Data:
09/249,675 12 February 1999 (12.02.99) US

(71) Applicant: AMGEN INC. [US/US]; One Amgen Center Drive, Thousand Oaks, CA 91320–1799 (US).

(72) Inventors: MARTIN, Frances, H.; 865 Fernhill Court, Newbury Park, CA 91320 (US). ELLIOTT, Steven, G.; 1040 Golden Crest Avenue, Newbury Park, CA 91320 (US).

(74) Agents: ODRE, Steven, M. et al.; Amgen, Inc., One Amgen Center Drive, Thousand Oaks, CA 91320–1799 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: GLYCOSYLATED LEPTIN COMPOSITIONS AND RELATED METHODS

(57) Abstract

The present invention relates to glycosylated leptin compositions and related methods. Included are glycosylated leptin proteins having a Stokes' radius allowing for improved properties, as well as glycosylated leptin proteins having selected sites for glycosylation, nucleic acids encoding such proteins, related host cells, vectors, processes for production, and methods of use of such compositions. Novel methods of producing glycosylated proteins are also provided. The glycolysated leptin protein can be used for preparing a pharmaceutical composition that can be used in the treatment of a human for a condition selected among obesity, diabetes and high blood lipid content.

SEQ ID NO: 6

```
<!--StartFragment-->RESULT 1
US-07-679-052A-2
; Sequence 2, Application US/07679052A
; Patent No. 5298400
;  GENERAL INFORMATION:
;    APPLICANT:  WHITFELD, Peter L.
;    APPLICANT:  RICHARDSON, Michael A.
;    APPLICANT:  BUNN, Clive L.
;    TITLE OF INVENTION:  RECOMBINANT PRODUCT
;    NUMBER OF SEQUENCES:  17
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  Foley & Lardner
;      STREET:  1800 Diagonal Road, Suite 500
;      CITY:  Alexandria
;      STATE:  Virginia
;      COUNTRY:  USA
;      ZIP:  22313-0299
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  Floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  PatentIn Release #1.0, Version #1.25
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/07/679,052A
;      FILING DATE:  19910506
;      CLASSIFICATION:  514
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  BENT, Stephen A.
;      REGISTRATION NUMBER:  29,768
;      REFERENCE/DOCKET NUMBER:  16786/147 CHAC
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  (703)836-9300
;      TELEFAX:  (703)683-4109
;      TELEX:  899149
;  INFORMATION FOR SEQ ID NO:  2:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  24 amino acids
;      TYPE:  AMINO ACID
;      TOPOLOGY:  unknown
;    MOLECULE TYPE:  peptide
;    FEATURE:
;      NAME/KEY:  Peptide
;      LOCATION:  1..24
;      OTHER INFORMATION:  /note= "Signal peptide from human
;      OTHER INFORMATION:  a-1-antitrypsin"
US-07-679-052A-2

  Query Match             100.0%;  Score 124;  DB 1;  Length 24;
  Best Local Similarity   100.0%;  Pred. No. 1.1e-09;
  Matches   24;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 MPSSVSWGILLLAGLCCLVPVSLA 24
              ||||||||||||||||||||||||
Db          1 MPSSVSWGILLLAGLCCLVPVSLA 24

<!--EndFragment-->
```

US005298400A

United States Patent [19]

Whitfeld et al.

[11] Patent Number: 5,298,400

[45] Date of Patent: Mar. 29, 1994

[54] **POLYNUCLEOTIDE CONSTRUCTS FOR SECRETED GLYCOSYLATED PLASMINOGEN ACTIVATOR INHIBITOR-2 (PAI-2)**

[75] Inventors: **Peter L. Whitfeld**, Glebe; **Michael A. Richardson**, Belrose; **Clive L. Bunn**, West Ryde, all of Australia

[73] Assignee: **Biotechnology Australia Pty. Ltd.**, New South Wales, Australia

[21] Appl. No.: **679,052**

[22] PCT Filed: **Sep. 4, 1990**

[86] PCT No.: **PCT/AU90/00396**

§ 371 Date: **May 6, 1991**

§ 102(e) Date: **May 6, 1991**

[87] PCT Pub. No.: **WO91/03556**

PCT Pub. Date: **Mar. 21, 1991**

[30] **Foreign Application Priority Data**

Sep. 5, 1989 [AU] Australia PJ6179

[51] **Int. Cl.**[5] **C12N 15/15**; C12N 15/03; C12N 15/06; C12P 21/02

[52] **U.S. Cl.** **435/69.8**; 435/69.2; 435/172.3; 435/240.1; 435/240.2; 435/320.1

[58] **Field of Search** 536/27, 23.5; 435/320.1, 69.2, 69.8, 240.1, 240.2, 172.3; 935/48

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,546,082 10/1985 Kurjan et al. 435/172.3

FOREIGN PATENT DOCUMENTS

0278696	8/1988	European Pat. Off. .
278696	8/1988	European Pat. Off. .
3713272	11/1988	Fed. Rep. of Germany .
3722673A1	1/1989	Fed. Rep. of Germany .
2611723	9/1988	France .
63-233789	9/1988	Japan .
85/00191	3/1986	World Int. Prop. O. .
87/00068	9/1987	World Int. Prop. O. .
WO87/05628	9/1987	World Int. Prop. O. .
WO87/06590	11/1987	World Int. Prop. O. .

OTHER PUBLICATIONS

Leicht et al., "Sequence homology and structural comparison . . . " Nature 297 pp. 655–659, 1982.

Luckow et al., "Trends in the Development of Baculovirus Expression Vectors", *Bio/Technology*, 6: 47–55 (1988).

Bishop et al., "Baculovirus Expression Vectors", *Advances in Gene Technology*, 1:55–72 (1990).

Fraser, "Expression of Eucaryotic Genes in Insect Cell Cultures", *In Vitro Cellular & Developmental Biology*, 25(3), Part 1, 225–235 (1989).

R. Ye, et al., "Mammalian Protein Secretion Without Signal Peptide Removal", *Journal of Biological Chemistry*, vol. 263, No. 10, 1988, pp. 4869–4875.

R. Ye, et al., "cDNA Cloning and Expression in *Escherichia coli* of a Plasminogin Activator Inhibitor from Human Placenta", Journal of Biological Chemistry, vol. 262, No. 8, 1987, pp. 3718–3725.

E. Lawrence, "Henderson's Dictionary of Biological Terms", 10th edition, 1989, p. 315.

S. Ali, et al., "Characterisation of the Alleles encoding ovine β-lactoglobulins A and B", GENE, vol. 19, 1990, pp. 201–207.

(List continued on next page.)

Primary Examiner—Robert J. Hill, Jr.
Assistant Examiner—Marianne Porta Allen
Attorney, Agent, or Firm—Foley & Lardner

[57] **ABSTRACT**

This invention relates to PAI-2 and its expression as a recombinant molecule in eukaryotic cell lines as a glycosylated secreted molecule, to the constructs expressing it, to host cells expressing it, to compositions comprising it, to methods of treatment, prophylaxis and diagnosis using it and to antibodies raised against it. The invention also provides a 414 amino acid form of PAI-2 wherein the N-terminal methionine residue is deleted, a 60 kD glycosylated secreted recombinant form of PAI-2 and compositions and methods using these molecules. The invention further relates to a novel synthetic signal peptide.

11 Claims, 19 Drawing Sheets

SEQ ID NO: 8

```
<!--StartFragment-->RESULT 5
5521070-2
;Patent No. 5521070
;     APPLICANT: MEULIEN, PIERRE
;     TITLE OF INVENTION: DNA SEQUENCE CODING FOR HUMAN FACTOR
;IX OR A SIMILAR PROTEIN, EXPRESSION VECTOR, TRANSFORMED CELLS,
;METHOD FOR PREPARING FACTOR IX AND CORRESPONDING PRODUCTS OBTAINED
;     NUMBER OF SEQUENCES: 6
;     CURRENT APPLICATION DATA:
;       APPLICATION NUMBER:  US/08/209,489
;       FILING DATE: 14-MAR-1994
;     PRIOR APPLICATION DATA:
;       APPLICATION NUMBER: 970,966
;       FILING DATE: 03-NOV-1992
;       APPLICATION NUMBER: 433,276
;       FILING DATE: 08-NOV-1989
;SEQ ID NO:2:
;       LENGTH: 461
5521070-2

  Query Match             96.2%;  Score 228;  DB 7;  Length 461;
  Best Local Similarity   97.8%;  Pred. No. 1.2e-25;
  Matches   45;  Conservative    0;  Mismatches    1;  Indels    0;  Gaps    0;

Qy          1 MQRVNMIMAESPGLITICLLGYLLSAECTVFLDHENANKILNRPKR 46
              |||||||||||||||||||||||||||||||||||||||||||| ||
Db          1 MQRVNMIMAESPGLITICLLGYLLSAECTVFLDHENANKILNRVKR 46
<!--EndFragment-->
```

SEQ ID NO: 9

US005521070A

United States Patent [19]

Meulien

[11] Patent Number: 5,521,070

[45] Date of Patent: May 28, 1996

[54] **DNA SEQUENCE CODING FOR HUMAN FACTOR IX OR A SIMILAR PROTEIN, EXPRESSION VECTOR, TRANSFORMED CELLS, METHOD FOR PREPARING FACTOR IX AND CORRESPONDING PRODUCTS OBTAINED**

[75] Inventor: **Pierre Meulien**, Strasbourg, France

[73] Assignee: **Transgene S.A.**, Courbevoie, France

[21] Appl. No.: **209,489**

[22] Filed: **Mar. 14, 1994**

Related U.S. Application Data

[63] Continuation of Ser. No. 970,966, Nov. 3, 1992, which is a continuation of Ser. No. 433,276, Nov. 8, 1989.

[30] **Foreign Application Priority Data**

Nov. 9, 1988 [FR] France 88 14635

[51] **Int. Cl.**[6] **C12N 15/00**; C12P 21/06; C07K 14/00; C07H 21/04

[52] **U.S. Cl.** **435/69.1**; 435/172.3; 435/320.1; 530/350; 530/381; 536/23.5; 536/23.2

[58] **Field of Search** 435/69.1, 320.1, 435/172.3; 536/23.2, 23.5; 530/350, 381

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,760,025	7/1988	Estell	435/222
4,770,999	9/1988	Kaufman	435/68

OTHER PUBLICATIONS

Pavirani et al. Biotechnology 5:384, 1987.
Kurochi et al PNAS 79: 6461, 1982.
Bentley et al Cell 45: 343, 1986.
DiScipio et al Biochemistry 16(4): 698, 1977.
de la Salle et al Nature 316: 268, 1985.

Primary Examiner—Suzanne E. Ziska
Attorney, Agent, or Firm—Burns, Doane, Swecker & Mathis

[57] **ABSTRACT**

The present invention relates to a novel DNA sequence coding for factor IX or a similar protein, corresponding to a prosequence and to mature factor IX or the mature similar protein. According to the invention, position (−3) in the prosequence is occupied by a codon coding for valine, arginine, lysine, threonine or serine, and/or the first codon of the sequence coding for the mature protein codes for an alanine.

16 Claims, 3 Drawing Sheets

SEQ ID NO: 9